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The Chick *Brachyury* Gene: Developmental Expression Pattern and Response to Axial Induction by Localized Activin

ANDREAS KISPERT,*[,1] HUBERT ORTNER,*[,2] JONATHAN COOKE,† AND BERNHARD G. HERRMANN*[,2,3]

*Max-Planck-Institut für Entwicklungsbiologie, Abteilung Biochemie, Postfach 2109, D-72011 Tübingen, Germany; and †National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England

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The mouse *Brachyury* gene (*T*) is required in notochord differentiation and posterior mesoderm formation during axial development. We have isolated the chick homologue of *T*(*Ch-T*) and determined its putative protein sequence and expression pattern during embryogenesis. *Ch-T* is expressed in the epiblast close to and within the primitive streak, in early migrating mesoderm and in the notochord. In later stages *Ch-T* expression is found in the tail bud and in the entire notochord. The notochord expression ceases in an anterior–posterior wave when the formation of the body anlage is completed. This pattern is consistent with those reported for the expression of the mouse *T* gene and the *T* homologues of *Xenopus laevis* and zebrafish, suggesting that the mechanisms of embryonic pattern formation are highly conserved in all vertebrates. The N-terminal half of *Ch-T* shows a very high degree of sequence identity with the corresponding region of mouse T which has DNA-binding activity, and with the N-terminal half of *Xenopus* (Xbra) and zebrafish (Ntl) T protein. Finally, we have analyzed the effects of activin A on *Ch-T* induction and axis formation. Localized activin A treatment of prestreak blastoderms results in ectopic *Ch-T* expression that correlates with formation of second primitive streaks or with repositioning of the site of single streak origin (Cooke *et al.*, 1994). These results strengthen the previous evidence that *Brachyury* activation is an early response to axis-inducing signals *in vivo*.

INTRODUCTION

The mouse *Brachyury* gene (*T*) has been shown to be required in notochord differentiation and posterior mesoderm formation (Chesley, 1935; for review see Herrmann and Kispert, 1994). Embryos lacking *T* undergo anterior development, including anterior mesoderm formation and heart development, but lack the trunk and tail. The allantois, the precursor of the umbilical cord, is severely reduced, resulting in embryonic death at the 11th day of gestation (Glücksohn-Schöenheimer, 1944). *T/T* embryos form a notochordal plate, but are not capable of notochord differentiation. At the 9th day of gestation mesoderm formation in the primitive streak is arrested.

The mouse *T* gene is expressed in all cells ingressing through the primitive streak (Wilkinson *et al.*, 1990; Herrmann, 1991; Kispert and Herrmann, 1994). Expression commences in the ectodermal aspect of the primitive streak. Soon after ingression of migrating mesoderm and endoderm cells, *T* expression becomes undetectable in these cells. In the notochord, however, T protein is observed until the end of embryonic body formation.

Recently, the isolation and expression of the *Xenopus* (*Xbra*) and Zebrafish (*Zf-T*) homologues of *T* have been reported (Smith *et al.*, 1991; Schulte-Merker *et al.*, 1992). Both show a high degree of identity within the N-terminal half of the T protein. The expression pattern of these homologues is also conserved, such that *Xbra* is expressed in the marginal zone and notochord and *Zf-T* is expressed in the germ ring and notochord. The marginal zone of *Xenopus* and the germ ring of zebrafish embryos generate the mesoderm and endoderm. *Xbra* and *Zf-T*, like mouse *T*, are transiently expressed in nascent and migrating mesoderm and endoderm, and continuously in the notochord. The conservation of *T* expression in vertebrates argues for a common patterning mechanism of all vertebrate embryos.

In contrast to vertebrates the ascidian (a lower chordate) embryo shows *T* expression only in notochord cells, but not in mesenchyme, although the notochord lineage is derived from cells with the potential of producing notochord, muscle, mesenchyme, and spinal cord

[1] Present address: Harvard University, The Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138.

[2] Present address: Max-Planck-Institut für Immunbiologie, Abt. Entwicklungsbiologie, Stübeweg 51, D-79108 Freiburg, Germany.

[3] To whom correspondence should be addressed. Fax: 49-7615-108 569.

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(Yasuo and Satoh, 1993). *T* expression is switched on as soon as a cell is destined to produce notochord only. This suggests that the more ancient function of *T*, in evolutionary terms, is to specify notochord identity.

The analysis of the zebrafish mutant *no tail* (*ntl*) showed that the function of *T* in vertebrate embryogenesis is also conserved (Halpern *et al.*, 1993). *ntl* has been identified as a mutation in the zebrafish *T* gene *Zf-T* (Schulte-Merker *et al.*, 1994). Like *T/T* mouse embryos, *ntl* homozygotes undergo anterior development, but lack the posterior trunk. *ntl/ntl* embryos form a notochord precursor (the equivalent of the head process or notochordal plate in mouse and chick) which fails to differentiate into the notochord and are arrested in mesoderm formation posterior to approximately the 19th somite.

The T protein most likely functions as a transcription factor (Kispert and Herrmann, 1993). It has been shown to be localized to the nucleus and to bind to a specific target recognition sequence *in vitro*. The highly conserved N-terminal half of T protein, termed the T domain, confers the DNA-binding activity.

We have isolated the chick *T* homologue (*Ch-T*). It has a high degree of identity to the other vertebrate T proteins within the T domain and is expressed in the primitive streak and in the notochord. Therefore, it behaves like the *T* gene of other vertebrates. We have used *Ch-T* as a marker to analyze the effect of ectopic activin A on axial development in the chick embryo. In some cases activin A is capable of inducing a second primitive streak in early embryos. In most cases it repositions the origin of the host's single primitive streak instead, such that it forms adjacent to the site of the activin-releasing graft. New expression of *Ch-T* is an early indication of such second or repositioned streak sites, adding to the evidence from elsewhere that *T* expression is an early, and probably immediate, response to mesoendoderm-inducing signals in normal development.

MATERIALS AND METHODS

cDNA Isolation and Sequencing

A cDNA library of Embryonic Day 2–3 chick notochord was constructed and directionally cloned in the plasmid pMT21 (Cliff Hume and Jane Dodd, personal communication). The library was screened with a DNA fragment, encoding amino acid residues 27–229, derived from the mouse cDNA me75 (Herrmann *et al.*, 1990) according to a procedure by Herrmann *et al.* (1987) developed for the screening of cosmid libraries. A number of hybridizing colonies were identified, of which 10 clones were purified and analyzed. Two cDNA clones were prepared for sequencing by controlled nested deletion from

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T     MSSPGTESAG KSLQYRVDHL LSAVESELQA GSEKGDPTER ELRVGLEESE LWLRFKELTN EMIVTKNGRR
Ch-T  -G--..-D-- -APA------ ---------- ---------- ----A--DG- ---------- ----------
Xbra  -.-A.---CA -NV------- -----N---- ---------K --K-S---RD --T------- ----------
Ntl         M-.A S-PDQ-L--- -------F-K ------AS-- DIKLS--DA- --TK------ ------T---

T     MFPVLKVNVS GLDPNAMYSF LLDFVTADNH RWKYVNGEWV PGGKPEPQAP SCVYIHPDSP NFGAHWMKAP
Ch-T  -------S-- ---------- -----A--G- ------C--- ---------- ---------- ----------
Xbra  -------SM- ---------TV -----A---- ---------- ---------- ---------- --------D-
Ntl   -----RAS-T ---------V -----A---N ---------- --------S- ---------- ----------

T     VSFSKVKLTN KLNGGGQIML NSLHKYEPRI HIVRVGGPQR MITSHCFPET QFIAVTAYQN EEITALKIKY
Ch-T  ---------- ---------- ---------- ---------- -----S---- ---------- ----------
Xbra  ---------- -M-------- ---------- -------T-- -----S---- ---------- ---------H
Ntl   --------S- ---------- ---------- ---K---I-K --S-QS---- ---------- ---------H

T     NPFAKAFLDA KERNDHKDVM EEPGDCQQPG YSQWG.WLVP GAGTLCPPAS SHPQF.GGSL SLPSTHGCER
Ch-T  ---------- --------M- --A--N--S- ---L-S--I- -T-A-----N P-S--.-AP- --SPA-S---
Xbra  ---------- -----Y--IL D-GI-S-HSN F--L-T--I- NG-S--S-NP .-T--.-AP- --S-P-----
Ntl   ---------- ---S---E-P DHST-N--S- ---L-G-FL- SN-P.MG-S- -P---N-APV H.S-GSY---

T     YPALRNHRSS PYPSPYAHRN SSPT.YADNS SACLSMLQSH DNWSSLGVPG HTSMLPVSHN ASPPTGSSQY
Ch-T  -SP------A ---N--T--- N---A-T--- ----P----- ---------T --T---M--S TGTA-S----
Xbra  -SS------A ------T--- N--NNL---- ---------- ----T-QM-A --G---M--S TGT-PP----
Ntl   -SS-----AA ----H-S--S TTTNN-M--- -GS-A...-- -S--A-QI-N SSG-GTLA-T TNTTSNT---

T     PSLWSVSNGT ITP.GSQTAG VSNGLGAQFF RGSPAHYTPL THTVSAATSS SSGSP.MYEG AATVTDISDS
Ch-T  --------S- ---.AP-SS- M----SS--L ----V---A- P-P-T-T--T -...-.L-D- G-PA.-LP--
Xbra  --------SA ---V.--SG- IT--ISS-YL L--TP--SS- S-A-PSPSTG -...-.L--H G-Q.-E-AEN
Ntl   ------AGT- L--S--ASGS ITG--TS--L ---SMS-SG- -SSLPVSSP- -MYD-GLS-V GVGDAQFES-

T     QYDT.AQSLL IASWTPVSPP SM
Ch-T  ---AS-HTR- ASM---IT-- --
Xbra  ---VT-H-R- SST----A-- -V
Ntl   .I...-..R- T---A--AQ. -Y
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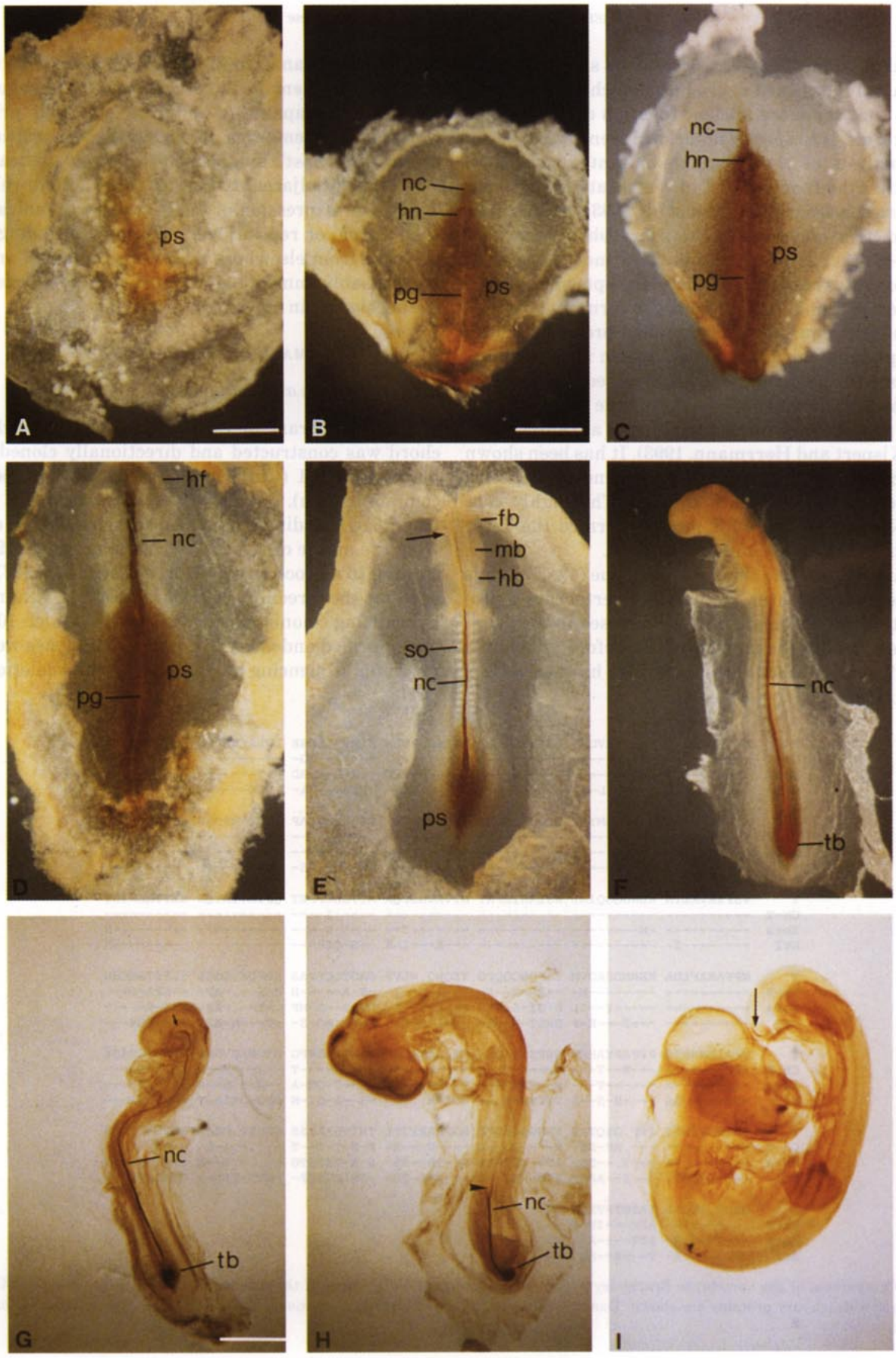
FIG. 1. Comparison of the vertebrate Brachyury proteins. The amino acid sequences of the mouse (T), chick (Ch-T), *Xenopus* (Xbra), and zebrafish (Ntl) Brachyury proteins are shown. Dashes indicate amino acid identity with mouse T, dots indicate gaps introduced for optimal alignment.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either the 5′ or the 3′ end using the Erase a Base kit purchased from Promega and sequenced using either the Autoread sequencing kit of Pharmacia and the ALF automatic sequencer (Pharmacia) or the T7 sequencing kit (Pharmacia) according to the supplier's directions.

Experimental Induction of Second Streaks

Grafts of pelleted, transfected CHO cells secreting active activin A (courtesy of G. Wong) were grafted into prestreak blastoderms as described previously (Cooke *et al.*, 1994). Wholemount immunocytochemistry on blastoderms having reached stages 2–5 following such grafting operations was performed according to Kispert and Herrmann (1994), using the antiserum α-TN$_{1-123}$ which was directed against the mouse T protein.

Histology

Following the immunocytochemical detection of T protein, wholemount embryos were embedded using the Polaron Embedding kit (Bio-Rad Polaron Instruments) according to the supplier's directions, mounted, and sectioned with a glass knife.

RESULTS AND DISCUSSION

The Sequence of Ch-T

A cDNA library (kindly provided by T. Jessel) prepared from RNA of Embryonic Day 2–3 chick notochord was screened by hybridization with a fragment of the mouse *T* cDNA me75 (Herrmann *et al.*, 1990) encoding the conserved N-terminal protein half. Several positive clones were analyzed and one clone, pch10, was sequenced completely. It contains almost the complete sequence of the chick *T* (*Ch-T*) transcript, with a poly(A) tail and the entire protein coding region. The open reading frame codes for a protein of 433 amino acid residues (data not shown).

A comparison of the predicted protein sequence of *Ch-T* with the previously published sequences of mouse *T* and the *T* homologues of *Xenopus laevis* (Smith *et al.*, 1991) and the zebrafish *Brachydanio rerio* (Schulte-Merker *et al.*, 1992) shows a high degree of identity within the N-terminal half of these proteins, the T domain (amino acids 1–229 in mouse) which confers DNA-binding activity (Kispert and Herrmann, 1993). The T domains of mouse and chick are 96% identical (Fig. 1).

The Expression Pattern of Ch-T

To verify that the cDNA pch10 was derived from the chick *Brachyury* homologue as opposed to a similar but distinct gene, we analyzed the RNA expression pattern of the corresponding gene in wholemount embryos using antisense RNA transcribed from pch10 (not shown). The expression pattern obtained is consistent with the data reported on mouse embryos (Wilkinson *et al.*, 1990; Herrmann, 1991) and indistinguishable from the protein expression pattern in the chick, described below, analyzed with an antiserum directed against the N-terminus of the mouse T protein, α-TN$_{1-123}$ (Kispert and Herrmann, 1994). Therefore, taking into account the expression pattern and the sequence conservation to mouse *T*, pch10 must be derived from the chick *Brachyury* homologue.

Ch-T expression commences with the beginning of primitive streak formation and continues until the embryonic body anlage is completed (Fig. 2). During streak formation (stage 2, Hamburger and Hamilton, 1992) there is first an intermixture of *Ch-T*-positive and *Ch-T*-negative cells detectable in the epiblast within a posterior triangular region (not shown). Soon, however, all cells are *Ch-T*-positive within this region except for an

FIG. 2. Expression of Ch-T protein in wholemount chick embryos. Embryos were immunostained as wholemounts with α-TN$_{1-123}$ serum and photographed without (A–F) or after (G–I) clearing in Murray's clearing agent. Staging (H&H) is according to Hamburger and Hamilton (1992). (A) H&H 3, intermediate primitive streak; *Ch-T* expression marks the primitive streak, extending from the posterior blastoderm margin to approximately the middle of the *area pellucida*. (B) H&H 5, the primitive streak is fully extended, the node, primitive groove, and head process (notochordal plate) are visible. Notochordal plate and primitive streak are strongly *Ch-T*-positive. (C) H&H 5. (D) H&H 6, head fold stage; the node regresses and axial development proceeds. *Ch-T* expression anterior to the node is restricted to the notochord. The posterior end of the primitive streak lies in the *area opaca;* it contains *Ch-T*-positive cells probably forming extraembryonic mesoderm. (E) H&H 10, 10 somite stage; the anterior limit of the notochord marks the boundary between forebrain and midbrain (arrow). (F) H&H 14; 22 somite stage. The primitive streak has disappeared and been replaced by the tail bud. In plates E and F the anterior part of the notochord appears weakly stained. This is due to the fact that it is obscured by brain tissue; however, there is no difference in expression detectable at the microscopic level, compared to more posterior notochord. (G) H&H 15; 27 somite stage. Staining of the notochord is getting weaker anteriorly, but is still visible up to the forebrain boundary (arrow). (H) H&H 21; Ch-T protein has become undetectable in most of the notochord, except for the caudal region and the tailbud; the arrowhead indicates the anterior limit of *Ch-T* in the notochord. (I) H&H 21; a small region of tail notochord is the last sign of *Ch-T* expression (arrow). A–D, dorsal view; E, ventral view; F–I, lateral view; anterior is always to the top. fb, forebrain; hb, hindbrain; hf, head fold; hn, Hensen's node; mb, midbrain; nc, notochordal plate (head process)/notochord; pg, primitive groove; ps, primitive streak; so, somite; tb, tail bud. Scale bars in A and B correspond to 550 μm and apply also to C–F; scale bar in G, 850 μm; also applies to H and I.

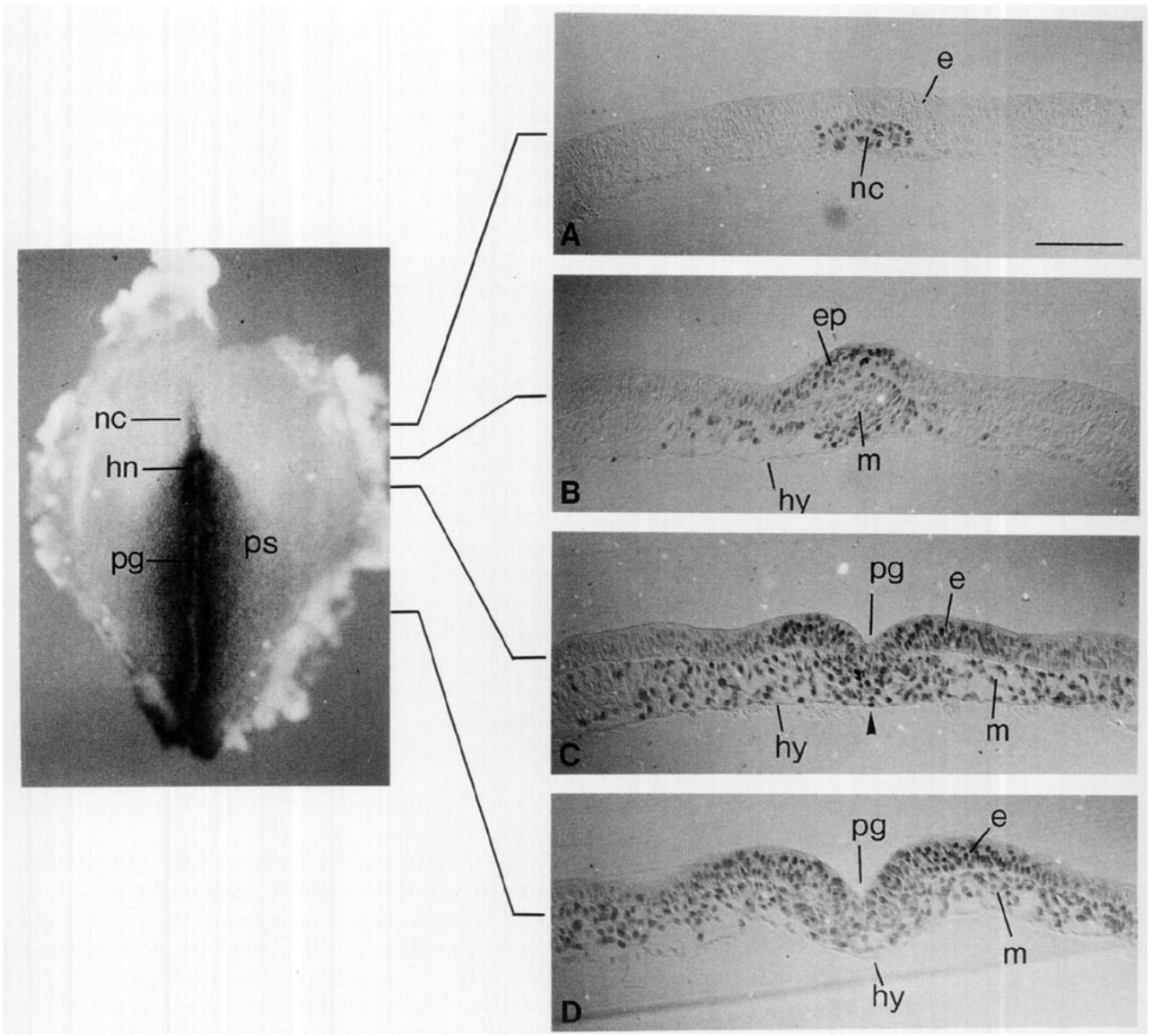


FIG. 3. *Ch-T* expression in a sectioned primitive streak stage chick embryo. A H&H stage 5 embryo was stained for Ch-T protein as a whole-mount preparation and serially sectioned transversely, but somewhat obliquely to the embryonic axis. The position of each section in the whole embryo is indicated. (A) A broad band of *Ch-T*-positive head process (notochordal plate) cells underlies the neuroectoderm anteriorly to Hensen's node. (B) Section through Hensen's node; the epiblast and mesoderm stain positive. (C and D) *Ch-T*-positive cells ingress through the primitive streak below the primitive groove. The arrowhead marks a *Ch-T*-positive endoderm cell. *Ch-T* staining is decreasing with increasing distance from the primitive streak, indicating down-regulation of *Ch-T* expression with time after ingression through the primitive streak. *Ch-T* expression in the epiblast is also restricted to the vicinity of the primitive streak. The scale bar in A corresponds to 80 μm and applies to plates A–D. e, ep, epiblast; hy, hypoblast; m, mesoderm; other abbreviations as in Fig. 2.

indefinite but narrow boundary zone. This development of a solid patch of expression over a period of about an hour, within epithelial tissue as opposed to among freely migrating cells, indicates that *Ch-T* is activated reliably in relation to position, rather than in a more widespread but scattered manner followed by selective aggregation of expressing cells. Ch-T protein is henceforth detectable throughout the length of the primitive streak and stays within the limits of the primitive streak as the node regresses (Fig. 2). *Ch-T* is later found in the tail bud. It is continuously expressed in the notochord from the first appearance of the head process to the completion of the embryonic body anlage. It disappears from the notochord in a rostrocaudal wave.

Sectioning of immunostained embryos demonstrated that *Ch-T* is found in the epiblast compartment of the

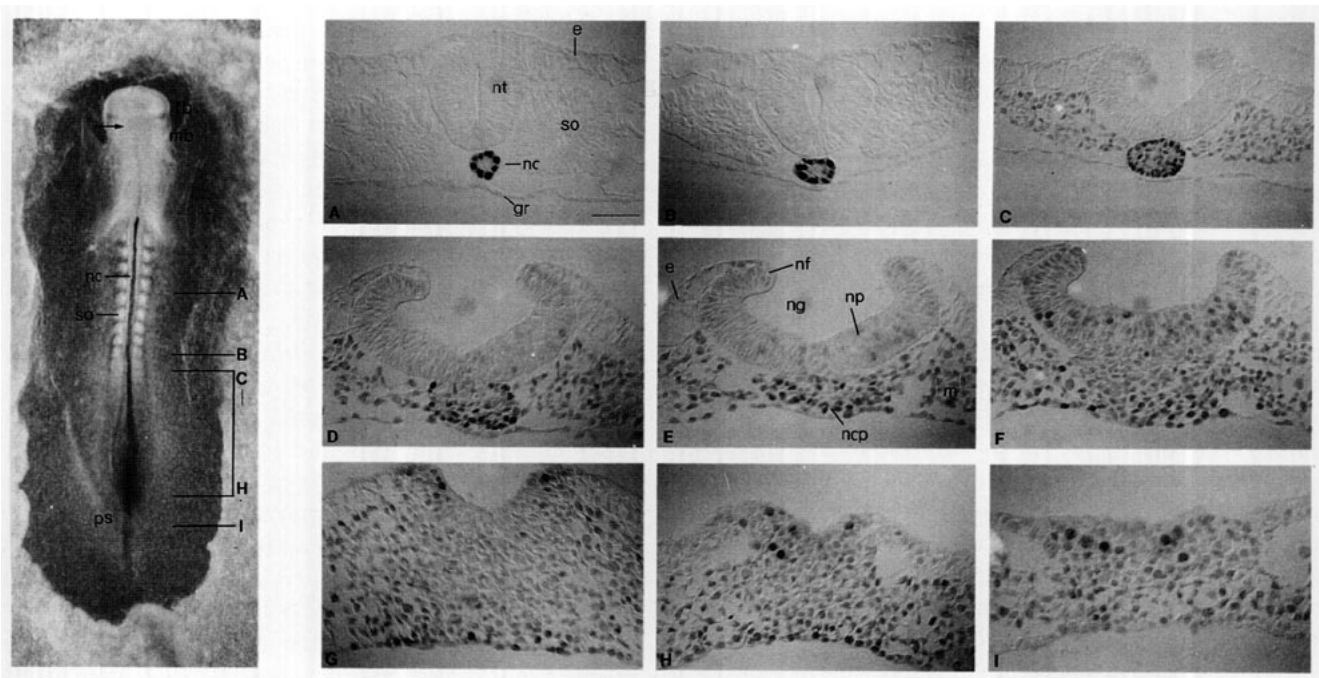


FIG. 4. *Ch-T* expression in a sectioned somite stage chick embryo. The embryo in the plate to the left was stained for Ch-T protein as a wholemount and serially sectioned transversely. It is shown in a ventral view; the positions of the sections are indicated at the plate margin. (A) Nuclear staining of notochord cells; the nuclei are located at the periphery. (B) Section through the segmental plate; the notochord is slightly larger than in A. (C–I) Progressively more posterior, that is, less differentiated regions of the embryo. Almost all cells, except for prospective epidermis, are *Ch-T*-positive initially to various degrees, but switch off *Ch-T* as they proceed with differentiation, except for the notochord cells which stain more intensely. Scale bar in A corresponds to 60 μm and applies also to plates B–I. e, epidermis; gr, roof of gut; m, mesoderm; ncp, notochordal plate; other abbreviations as in Fig. 2.

primitive streak and node (Fig. 3). As *Ch-T*-positive cells ingress through the primitive streak and move to paraxial and lateral positions, *Ch-T* becomes undetectable. Anterior to the node only the notochord cells stain *Ch-T*-positive. Node regression occurs by the streak shortening as anteriorly fated inner tissues emerge, leaving only more posteriorly fated cells within its epiblastic part. Thus, an increasingly elongated *Ch-T*-negative epiblast domain (including the future neural area) is left in the axis in front of the node, as the latter regresses.

In somite stage embryos Ch-T protein is detectable in most cells at the caudal end, including prospective gut endoderm and neuroectoderm (Fig. 4). As differentiation proceeds, that is in more and more anterior sections, *Ch-T* disappears from the endoderm and neuroectoderm first, while it is still detectable in the segmental plate and in the notochord, and finally is confined to the notochord. The nuclear localization of Ch-T protein is visible best in the notochord where the nuclei form a circle at the periphery. Transient expression of T protein in nascent endoderm and prospective neuroectoderm has also been reported for the somite stage mouse embryo (Kispert and Herrmann, 1994). The functional significance of this observation is unclear. But a unique correlation between *Ch-T* expression and a cellular commitment to mesoderm can be ruled out.

The pattern of *Ch-T* expression during tail development parallels the pattern during posterior trunk formation. Work in *Xenopus*, as well as in chick, has shown how in the tail bud the process of gastrulation effectively extends posteriorly (Gont *et al.*, 1993; Cooke, 1979; Schoenwolf, 1981). In the caudal tip of the tail bud *Ch-T* is expressed in all cells except for an ectodermal layer (Fig. 5). At the ventral side of the tail bud the ectodermal cells adjoin a plate of *Ch-T*-positive cells, probably nascent endoderm which is continuous with the mesenchymal cell mass within the tail bud. Mesoderm, endoderm, and prospective neuroectoderm initially express *Ch-T* transiently and lose it during differentiation, while in the notochord *Ch-T* expression stays on and becomes more prominent.

Different Vertebrates Show the Same Expression Pattern of Brachyury

A comparison of *Ch-T* expression in the chick (see above) with that of mouse *T* (Kispert and Herrmann, 1994) or *Xbra* in the frog (Smith *et al.*, 1991) and *Zf-T*

in the zebrafish (Schulte-Merker *et al.*, 1992), described previously, demonstrates a high conservation of the expression pattern of T in vertebrates. As pointed out here and elsewhere, in all four species T protein is transiently expressed in the primitive ectodermal cells (epiblast) destined to ingress (involute) and to form the mesoendoderm. Soon after ingression (involution) *T* expression is down-regulated in the differentiating mesoderm and endoderm cells. This transient expression suggests a function of *T* in establishing the mesoendoderm, rather than in mesoderm or endoderm differentiation. The expression of T protein in the notochord in all four species (not shown) provides a fixed point which allows us to relate *T*-positive regions of the embryo to the embryonic axes and to compare this pattern with the pattern in the other species. Such a comparison clearly shows that the primitive streak of mouse and chick are equivalent to the marginal zone of the frog and the germ ring of the zebrafish embryo. This similarity in the notochord and mesoendoderm formation strongly suggests a conservation in the pattern formation of all vertebrate embryos despite their quite different shapes.

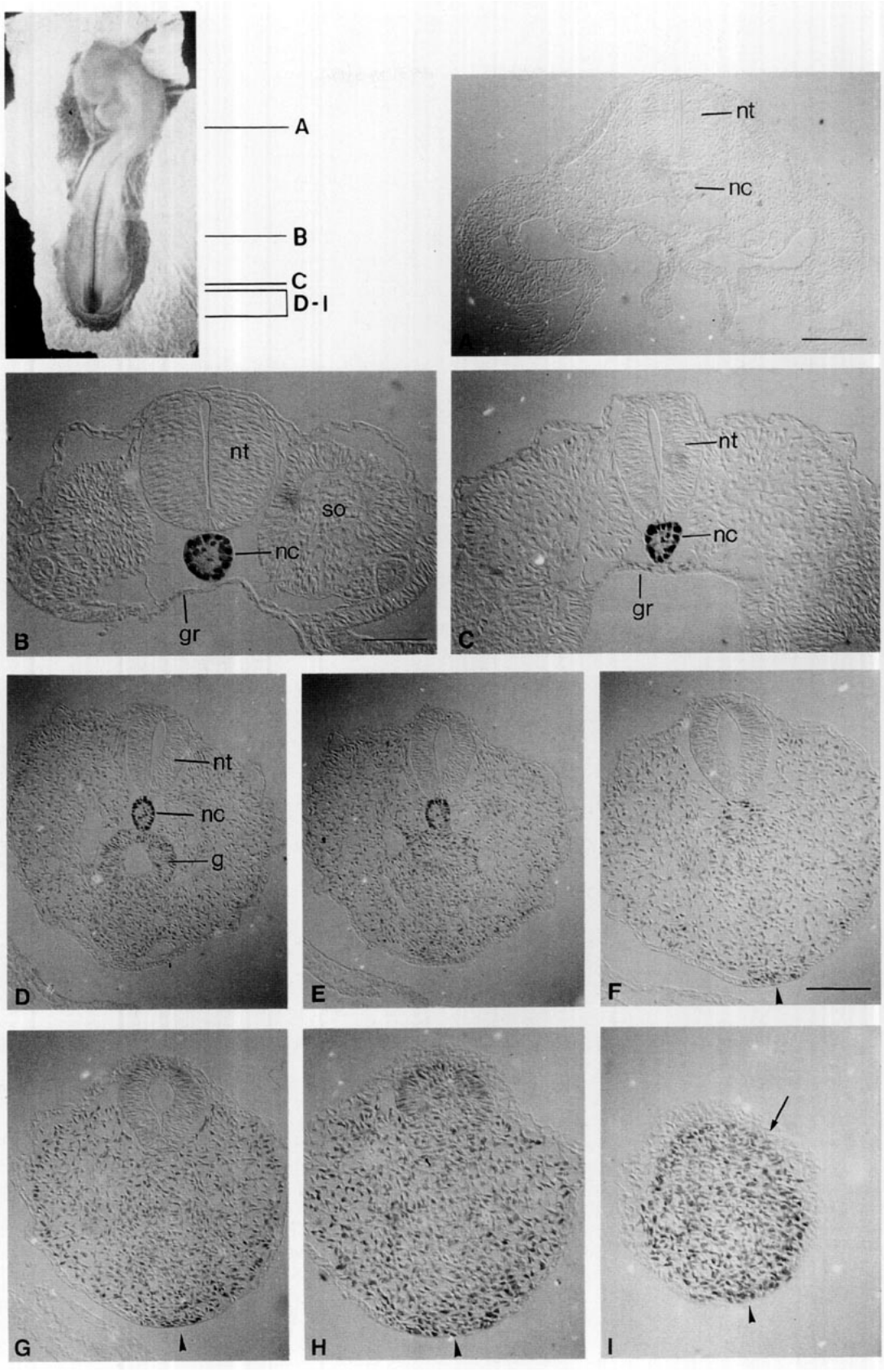
The Effect of Activin A on Ch-T Expression and Primitive Streak Formation

Activin A can act as a powerful mesoderm- and endoderm-inducing factor in the amphibian blastula (Smith *et al.*, 1989; Cooke, 1991; Jones *et al.*, 1993) and can mimic equivalent signaling processes in the bird blastoderm (Ziv *et al.*, 1992; Cooke *et al.*, 1994). Here we have analyzed *Ch-T* expression in relation to this experimental axial induction by activin A in the chick. CHO cells transfected with a human activin A construct and secreting the protein in active form, or control CHO cells, were pelleted and grafted into the annular zone lying just inside the area opaca of intact prestreak blastoderms (for details see Cooke *et al.*, 1994). The previous study showed that provided they are in place well before the expected time of origin of the primitive streak, the experimental (but not control) grafts characteristically reorganize the host axial patterning in relation to their own position. Most frequently the site of streak origin, and thus the orientation of the body axis, is repositioned so as to lie adjacent to the graft side. Such streaks are formed almost entirely from cells other than those that would originally have entered the streak. Alternatively, the pattern of development becomes twinned, with one streak forming near the originally expected site and another adjacent to the graft. Activin released from the graft thus appears able to replicate, or mimic, signals normally emenating from a particular site in the blastoderm marginal zone and resulting directly or indirectly in streak initiation.

Blastoderms in which a profound doubling has been induced in the cell movements accompanying streak formation are difficult to process as immunocytochemical wholemounts sufficiently flat to photograph in one optical plane. The anti-Brachyury antiserum nevertheless shows that next to the experimental activin secreting graft, when the site of streak formation has been repositioned or when a second streak is in very early stages of formation, the earliest *Ch-T* expression is appropriately repositioned or twinned (Fig. 6). Graft sites themselves are often encased in strongly *Ch-T*-positive host cells. Regions of *Ch-T* synthesis at incipient second streak sites are seldom as compact and regularly shaped as the normal one and are notably delayed in development relative to the host-controlled axis (Fig. 6A). By contrast, repositioned single axes are much more similar, in regularity and tempo of development, to those in control-grafted blastoderms that were synchronous at operation (Fig. 6B). In blastoderms that have received a scattering of a few hundred disaggregated activin-secreting cells onto the central hypoblast at prestreak stages, a large central disc-shaped area becomes *Ch-T*-positive, but without streak formation, when controls reach midstreak stages (not shown).

Taken together, these observations support the idea, derived from work in *Xenopus* (Smith *et al.*, 1991), that *T* is an immediate early response gene to mesoendoderm-inducing signals. They suggest that normal axis formation requires appropriate localization of the inducer source, perhaps to give a gradation of signal level, whether this signal is the natural inducing ligand or a related one that is able experimentally to substitute for it (Gurdon *et al.*, 1994). The relative time delay in onset

FIG. 5. *Ch-T* expression in a sectioned tail bud stage chick embryo. The embryo shown on the upper left plate was stained for Ch-T protein as a wholemount and sectioned transversely. The positions of the sections are indicated at the right margin. (A) *Ch-T* staining has disappeared from the notochord in the hindbrain, but is still strong in the trunk (B and C). (D–I) In the tail bud region weak *Ch-T* expression is detected in all mesodermal cells and in the gut. The notochord stains strongly. The neural tube is also weakly *Ch-T*-positive as it emerges from the mass of tail bud cells (G and H). Ventrally in the tail bud there is a plate of *Ch-T*-positive cells (marked by an arrowhead in F–I), probably representing endoderm, which is continuous with the mesenchymal cell mass within the tail bud, but integrated in the epidermal cell layer which is *Ch-T*-negative (indicated by an arrow in I). Scale bar in A corresponds to 110 μm; in B, 65 μm, also applies to C–E and G–I; in F, 80 μm. g, gut; gr, roof of gut; nc, notochord; nt, neural tube; so, somite.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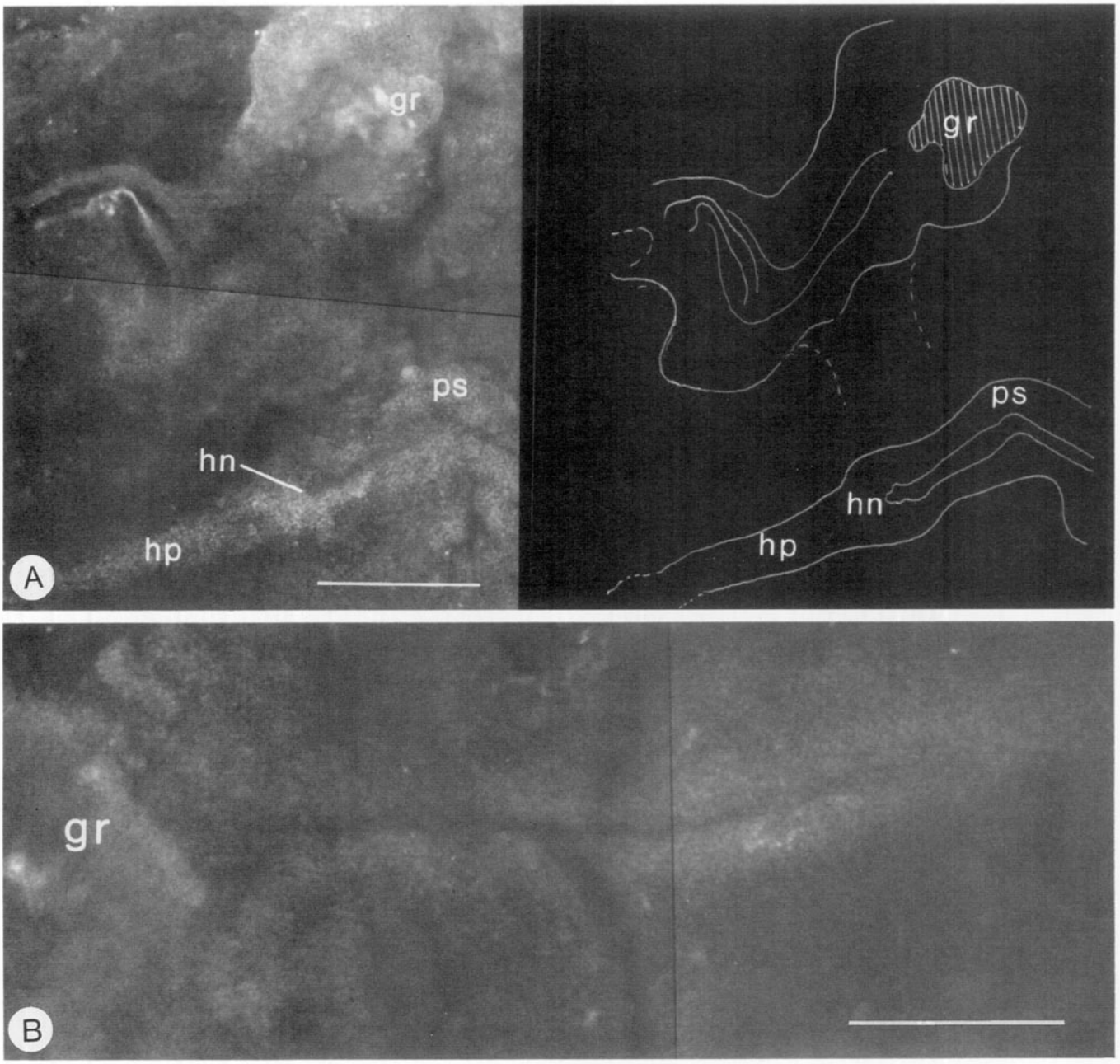

FIG. 6. *Ch-T* expression in a second or a repositioned axis, respectively, following placement of an activin-secreting graft. (A) *Ch-T* expression at an early stage of development of a twinned blastoderm pattern following an experimental "activin" CHO graft, seen heavily covered with positive host cells near top right. Anterior part of the "host" axis is seen in lower half of frame with Hensen's node and head process bent sharply away from graft region, as frequently observed. A second area of epiblast *Ch-T* positivity, resembling the normal very early streak, is related to the graft. The developmental stage disparity between "host" and graft-induced axial patterns is normal for second (as opposed to single repositioned) streaks. An explanatory tracing accompanies the photograph. (B) *Ch-T* expression in axis developing from a site opposite the "expected" one, next to an experimental graft of activin-secreting CHO cells (gr), seen to be covered in *Ch-T*-positive host cells at left. Thus, this blastoderm was photographed with the original presumptive site of streak origin at right as in (A). While blastoderm is of same age as in (A), the streak is of a somewhat earlier stage. Folds and bifurcations as seen are frequent in such ectopically induced streaks. hn, Hensen's node; ps, streak; hp, head process (notochordal plate) of "host" axis. gr, position of graft. Scale bar, 350 μm.

of *Ch-T* synthesis, when reorganization into two streak sites has occurred due to a grafted localized activin source, is similar to the relative delay in reaching later, anatomically defined stages, which has already been observed for such twinned axes (Cooke *et al.*, 1994). This suggests that the delay incurred in these early reorganizations is one in the initiating steps of gastrulation or axis establishment, involving signals and cell responses that lead up to—rather than result from—T expression, with subsequent development of the once-established axial patterns occurring on a normal schedule.

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